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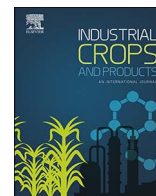
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Chemical characterization of the essential oil compositions and antioxidant activity from Iranian populations of *Achillea wilhelmsii* K.Koch

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ABSTRACT

To evaluate the chemical composition and the antioxidant activity of the essential oils from Iranian yarrow (*Achillea wilhelmsii* K.Koch), twenty accessions were collected from their natural habitats throughout southwest Iran. Essential oils were obtained by using a Clevenger-type apparatus, and were then chemically characterized by gas chromatography (GC) and gas chromatography with mass spectrometry (GC–MS) methods. Essential oil yields ranged from 0.14% to 0.44% (w/w) and 54 components were identified in the samples. The main components were found to be chrysanthenone (38.8%), *trans*-carveol (27.5%), linalool (26.1%), neoiso-dihydrocarveol acetate (25.2%), camphor (19.9%), filifolone (19.7%), 1,8-cineole (16.7%), borneol (13.6%), α -pinene (11.8%), *trans*-piperitol (11.7%), (*E*)-caryophyllene (11.2%), (*E*)-nerolidol (10.8%), and lavandulyl acetate (10.0%). The volatile composition of the yarrow accessions was analyzed using hierarchical cluster analysis. The yarrow populations fell into five chemotypes based on essential oil constituents. The antioxidant activity was evaluated using DPPH radical sequestering method ($IC_{50} = 129$ –372 mg/ml). Results showed a significant variation among accessions with regard to quality and quantity as well as biological activity of essential oils due to genetic makeup, environmental specifications, physiological conditions of the sampled plants and interaction between them. The current research provided new insights into the chemical variation of this species together with a possible application of its essential oil as a natural preservative agent in food and pharmaceutical industries.

1. Introduction

Iran supports a great share of plant species and countless natural habitats characterized by many unique plants and centers of local endemisms. The genus *Achillea* L. belongs to Asteraceae family and encompasses 130 species, 19 of which wild growing in Iran. *Achillea wilhelmsii* K.Koch (also known as *Achillea santolinoides* subsp. *wilhelmsii* (K.Koch) Greuter) is one of the widespread species of this genus in Iran (Rechinger, 1972). In the Iranian traditional medicine the plant is widely used for treating gastrointestinal disorders and as carminative (Asgary et al., 2000). Scientific evidences supported several therapeutic effects such as antihypertensive, antitumor, antimicrobial and vagolytic (Asgary et al., 2000; Tosun et al., 2004; Niazmand et al., 2010; Ali et al., 2011). Phytochemical characterization of *A. wilhelmsii* has been the subject of a very few studies, the majority of which focused on restricted geographical areas and the most abundant constituents have been reported to be camphor, linalool, borneol, carvacrol, *p*-cymene, 1,8-cineole, and thymol (Afsharypour et al., 1996; Javidnia et al., 2004;

Majnooni et al., 2013; Kazemi and Rostami, 2015).

Medicinal plants produce antioxidant compounds, which defend cells against degenerative effects of reactive oxygen species produced during oxidative stress and metabolism. Antioxidants are molecules that scavenge free radicals and reduce/prevent their damages. Therefore, the identification of natural antioxidants as preservative agents plays a pivotal role for the food, cosmetic and pharmaceutical industry (Saleh et al., 2010). Today's application of essential oils as active components in foods, drinks and cosmetics industries is becoming prevalent. Therefore, it is very important to discover new natural sources of safe, effective, eco-friendly and affordable antioxidants. As only a few studies have tried to consider a wider geographical range of distribution, the need for more investigations on this subject is obvious. To address the information gap mentioned above, in the current study, the variation in the essential oil yield, chemical constituents and antioxidant activity among twenty Iranian populations of *A. wilhelmsii*, has been investigated. The essential oils were obtained by hydro-distillation and analyzed by GC and GC–MS. Furthermore, samples were

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Table 1

Geographical distribution and essential oil content of *Achillea wilhelmsii* K.Koch populations throughout southwest Iran.

Code	Origin	Latitude	Longitude	Elevation (m a.s.l.)	EO content (%)
A1	Soreshjhan	32° 34'	50° 41'	2195	0.25
A2	Borujen	31° 58'	51° 15'	2216	0.30
A3	Farokhshar	32° 16'	50° 58'	2093	0.33
A4	Amirabad	32° 03'	50° 44'	2022	0.40
A5	Dehrashid	31° 31'	51° 09'	1857	0.36
A6	Bardeh	32° 33'	50° 31'	2377	0.26
A7	Dimeh	32° 30'	50° 12'	2270	0.44
A8	Kohyan	31° 28'	50° 53'	1728	0.18
A9	Haydarabad	32° 21'	50° 22'	2451	0.25
A10	Dezak	32° 09'	50° 20'	1983	0.15
A11	Aboueshagh	31° 19'	51° 16'	2201	0.16
A12	Gandoman	31° 50'	51° 08'	2269	0.19
A13	Faradonbeh	32° 02'	51° 12'	2166	0.25
A14	Shahrekord	32° 20'	50° 49'	2067	0.38
A15	Dashtak	32° 09'	50° 26'	1986	0.30
A16	Roodbal	30° 06'	52° 03'	1956	0.18
A17	Hajatagha	32° 21'	51° 06'	1961	0.14
A18	Soodejan	32° 31'	50° 22'	2248	0.40
A19	Saman	32° 26'	50° 53'	2045	0.19
A20	Farsan	32° 14'	50° 35'	2022	0.39

assayed for antioxidant activity by the DPPH method.

2. Materials and methods

2.1. Plant materials

Plants were collected in full flowering stage from their natural habitats in southwest Iran (Table 1 and Fig. 1). The botanical identification was made by using the Flora Iranica (Rechinger, 1972). Herbarium vouchers are kept at Research Center of Agriculture and Natural Resources, Shahrekord, Iran under the voucher 1995-1084-642. Samples identification was made by two expert botanists (Shirmardi, Hamzeh Ali PhD., Research Center of Agriculture and Natural Resources, P.O. Box 415, Shahrekord, IRAN and Shahrokhi, Asghar; Education Organization, Chaharmahal and Bakhtiari Province, Shahrekord, Iran).

2.2. Extraction of essential oil

The shade dried aerial parts, including stems, leaves and flowers (50 g) were subjected to hydrodistillation using a Clevenger-type apparatus for 3 h to get the essential oil (EO). The EO was dried over anhydrous sodium sulfate and stored in sealed vials with Teflon septa at 4 °C until analysis.

2.3. Gas chromatography analysis

The oils were analyzed using a Thermoquest-Finnigan Trace GC instrument equipped with flame ionization detector (FID). A DB-5 (30 m × 0.25 mm i.d., film thickness 0.25 mm) capillary column was used. The oven temperature program was 60–250 °C at the rate of 5 °C/min and finally held isothermally for 10 min. The injector and the detector temperatures were set at 250 °C; helium was used as the carrier gas with a flow rate of 1.1 ml/min.

2.4. Gas chromatography-mass spectrometry analysis (GC-MS)

Gas chromatography-mass spectrometry analysis was performed using a Thermoquest-Finnigan gas chromatograph equipped with the same column (DB-5, 30 m × 0.25 mm i.d., film thickness 0.25 mm), coupled with a TRACE mass ion trap detector (Manchester, UK). The oven temperature program was 60–250 °C at the rate of 5 °C/min and finally held isothermally for 10 min. Helium was used as carrier gas

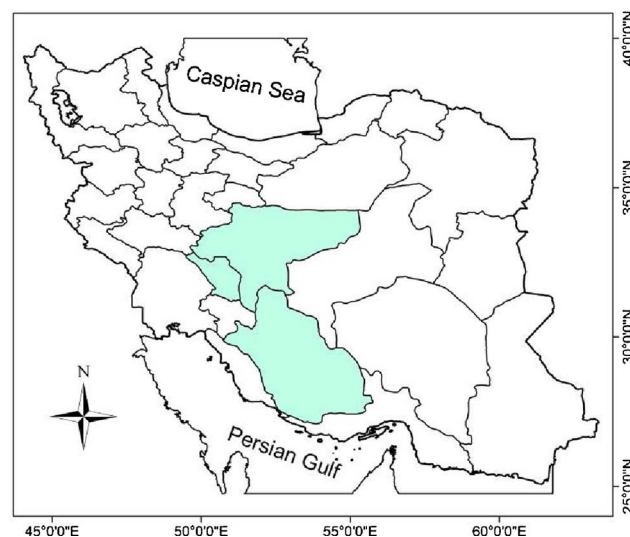
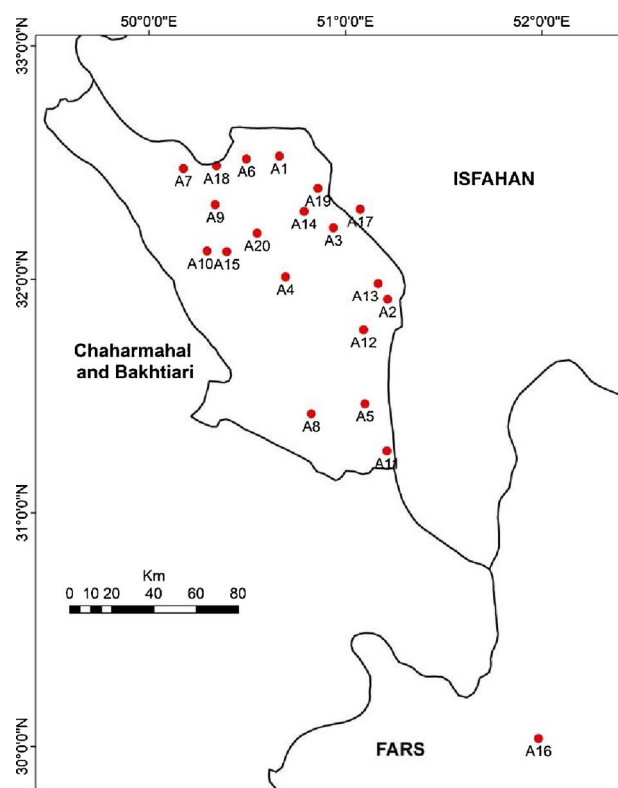


Fig. 1. Collection regions of the studied *Achillea wilhelmsii* K.Koch populations.

with an ionization voltage of 70 eV. Ion source and interface temperatures were 200 °C and 250 °C, respectively. Mass range was from 40 to 460 *m/z*. The chromatographic peaks were identified by the comparison of their Kovats retention indices with those of authentic standards. The calculation of the Kovats index was made based on a linear interpolation of the retention time of the homologous series of *n*-alkanes (C₇–C₂₄, Sigma). Data obtained were confirmed by the comparison of the mass spectra of each constituent either with those stored in the Wiley 7.0 and Adams mass spectral-retention index libraries (Adams, 2007) or with data published in the literature (Sandra and Bicchi, 1987; Davies, 1990).

2.5. Determination of DPPH radicals scavenging activity

In DPPH radical scavenging method, the free radicals 2,2-diphenyl-

1-picrylhydroxyl (DPPH) were used to find antioxidant activity of EOs. In a modified assay, 200 ml of a 100 mM solution of DPPH radical in methanol was mixed with 20 ml essential oil. After mixing, it was left for 30 min at room temperature. The DPPH radical inhibition was measured at 517 nm by using a microplate reader. The antioxidant activity (AOA) was given by:

$$100 - \left[\frac{(A)_{\text{sample}} - (A)_{\text{blank}}}{(A)_{\text{control}}} \times 100 \right],$$

Where A stands for the absorbance of the color formed in microplate wells, using DPPH as control (no EO), and blank consisted of only methanol. The IC₅₀ (mg/ml) of each sample (concentration in $\frac{\text{mg}}{\text{ml}}$ required to inhibit DPPH radical formation by 50%) was calculated. This was obtained by interpolation and using linear regression analysis. All experiments were conducted in triplicate, and measurements were reported as means \pm SD. For the calculation of these values, Microsoft Excel Software was used (Moein et al., 2007).

2.6. Statistical analysis

All statistical analyses were performed using Statistical Package for the Social Science (SPSS 16.0, SPSS Inc., USA) computer software. Specimen homogeneity was analyzed by hierarchical cluster analysis using Ward method based on the squared euclidean distance criterion.

3. Results and discussion

3.1. Essential oil yields and chemical compositions

The essential oil content demonstrates well the wide range of variability in *A. wilhelmsii* populations. The yield of EOs from *A. wilhelmsii* ranged from 0.14 to 0.44% (Table 1). This parameter was highest in Dimeh (A7) ecotype. Amirabad (A4), Soodejan (A18), Farsan (A20), and Dehrashid (A5) were other ecotypes with high content of EO. The population of Hajatagha (0.14%), Dezak (0.15%) and Aboueshagh (0.16%) presented the minimum content of EO. In previous studies, the essential oil yields from *A. wilhelmsii* were reported to be in the range 0.20–0.82% (Ghani et al., 2008; Kazemi and Rostami, 2015; Başer, 2016). In other studies, samples were selected from one region, whereas in this study samples were collected from a wider range of geographical areas. The difference between findings of the present investigation and other studies could be due to diversity in climatic conditions, genetic factors and interaction between them. In the context of this study, differences in geographical location and attendant climatic specifications, could affect chemical composition of a given taxon and hence bioactivity. Aside from phenotypic plasticity caused by geographical divergence, the appearance of numerous chemotypes recorded in this study reveals the importance of environmental parameters in physiological response of plants to external stimuli as well as their adaptive metabolism.

The composition of the essential oil was extremely variable among populations. Overall, 54 compounds were identified in the EOs from the twenty *A. wilhelmsii* populations accounting for 87.9–96.52% of the total components (Table 2). Results showed a relatively high variation in quantity and quality of components. Notably, chrysanthenone (0.2–38.8%), *trans*-carveol (tr-27.5%), linalool (0.8–26.1%), neoiso-dihydrocarveol acetate (tr-25.2%), camphor (0.8–19.9%), filifolone (tr-19.7%), 1,8-cineole (tr-16.7%), borneol (1.5–13.6%), α -pinene (tr-11.8%), *trans*-piperitol (tr-11.7%), (*E*)-caryophyllene (0.2–11.2%), (*E*)-nerolidol (tr-10.8%), and lavandulyl acetate (tr-10.0%) were found in abundance in the EOs of *A. wilhelmsii* populations (Table 3).

The highest amount of the above mentioned compounds were found in the oils from Aboueshagh (A11), Saman (A19), Roodbal (A16), Hajatagha (A17), Borujen (A2), Amirabad (A4), Farsan (A20), Gandoman (A12), Haydarabad (A9), Dashtak (A15), Aboueshagh

(A16), and Kohyan (A8) ecotypes, respectively. In general, the results obtained from this research were similar to previous studies (Afsharypour et al., 1996; Azadbakht et al., 2003; Ghani et al., 2008; Majnooni et al., 2013; Turkmenoglu et al., 2015; Ghasemi Pirbalouti, 2017). Chrysanthenone was found as one of the most compounds in all *A. wilhelmsii* ecotypes under study. In agreement with the findings of the present study, chrysanthenone was also reported by Ghasemi Pirbalouti (2017) as a major volatile constituent of *A. wilhelmsii* EOs. Carvacrol was reported as the main component of *A. wilhelmsii* in some previous studies in Iran (Javidnia et al., 2004; Dehghan and Elmi, 2015; Kazemi and Rostami, 2015). Likewise, we also detected this compound in all of the tested samples, but in minor quantities (0.1–1.2%).

Essential oils from the examined taxa were primarily rich in oxygenated monoterpenes accounting for 54.80–83.50% of the oils. The highest amount of oxygenated monoterpenes obtained in the sample collected from A17 and neoiso-dihydro carveol acetate (25.2%), chrysanthenone (15%), *trans*-carveol (8.7%), camphor (6%), linalool (5.9%), and *trans*-pinocarveol (5.7%) were major oxygenated monoterpenes. Soreshjan (A1) accumulated higher amount of monoterpenes hydrocarbons (19.80%) as compared with other ecotypes. α -Pinene was recognized as the main oil component (7.4%). In the A9 population, the sesquiterpenes hydrocarbons (12.20%) were present in higher abundance than in other areas, in which (*E*)-caryophyllene was the major component of this group (11.2%). Our data revealed that the highest amounts of oxygenated sesquiterpenes (17.20%) were found in the oil from A16 and (*E*)-nerolidol (10.81%) was found as the main oxygenated sesquiterpene in this ecotype. Oxygenated monoterpenes were the most abundant fraction in EOs from all ecotypes as well as in previous studies (Afsharypour et al., 1996; Azadbakht et al., 2003; Ghani et al., 2008; Majnooni et al., 2013; Turkmenoglu et al., 2015; Ghasemi Pirbalouti, 2017). In the present study, the samples were selected from different geographical regions whereas in previous studies the plants were collected from one geographical region. Therefore, the variability of chemical composition of the *A. wilhelmsii* essential oils may be influenced by various factors such as environmental conditions.

3.2. Hierarchical cluster analysis

To further explore the chemotaxonomic affinity and relationship amongst 20 ecotypes of *A. wilhelmsii*, the relative percentages of volatile constituents were submitted to hierarchical cluster analysis. The resulting dendrogram supports the idea that ecotypes of *A. wilhelmsii* can be distinguished based on their phytochemical profiles. Accordingly, the 20 yarrow accessions analyzed are distributed across five main groups, each representing a chemotype (Fig. 2). Based on results from dendrogram, a distinct separation observed between A16 and A4 ecotypes respect to the other ecotypes, having linalool and filifolone as major components, respectively. According to previous studies, the EO composition in the genus *Achillea* is highly variable due to various factors such as environmental conditions, ontogenic, and morphogenic differentiations and method applied for oil extraction (Kindlovits and Nemeth, 2012; Turkmenoglu et al., 2015). The differentiation of the A16 ecotype may be due to the climatic conditions of the sampling site that had a great geographical distance with the other areas.

Although A18, A19, A6, A12, and A17 ecotypes are different in terms of height, altitude, and latitude, they constituted a same group (Chemotype I); also chemotypes II and III were formed from ecotypes that did not have the same geographic conditions (Fig. 2). It has already been reported that the different environmental conditions of each location such as altitudes, solar exposition and soil types reflect the variability in EO compositions within a species. In addition, the differences in EO composition found for different geographical origins are also due to genetic differences and processing of the material after harvest (Figueiredo et al., 2008; Morshedloo et al., 2015).

Five populations formed chemotype I (A18, A19, A6, A17, and A12), as the EOs were rich in *trans*-carveol (8.7–27.5%), neoiso-

Table 2
Essential oil composition of *Achillea wilhelmii* K.Koch populations.

Component	RI ^a	RI	Lit. ^b	Accessions (%) ^c																				
				A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15	A16	A17	A18	A19	A20	
Tricyclene	925	926	0.3	0.1	0.1	0.2	0.1	0.1	tr ^e	0.2	0.1	0.1	tr	0.1	0.1	0.1	0.2	tr	tr	tr	tr	tr	0.2	
a-Pinene	935	939	7.4	5.4	3.2	4.6	2.6	3.1	3.1	2.1	8.4	11.8	7.5	7.6	6.6	2.4	7.2	tr	11.5	2.3	2.3	1.5	6.6	
Camphene	953	954	2.8	1.4	1.4	2.4	1.3	0.9	1.3	1.9	0.3	0.7	1.0	0.2	0.2	2.1	2.8	tr	0.1	0.4	0.3	0.2	3.7	
Sabinene	975	975	1.9	0.7	0.4	0.5	0.6	0.5	3.0	3.0	1.1	0.6	1.0	0.3	0.2	0.8	0.7	tr	0.3	0.2	0.6	0.4	2.6	
b-Pinene	980	979	1.1	0.4	0.1	0.3	0.1	0.2	0.5	0.5	0.5	0.3	0.3	0.2	0.8	0.4	0.3	tr	0.7	0.1	0.3	0.2	1.2	
r-Cymene	1026	1024	4.7	2.1	1.2	2.2	1.3	0.9	1.1	1.1	2.5	2.3	2.1	1.2	1.2	1.3	0.9	0.4	1.2	0.6	1.6	1.1	2.6	
1,8-Cineole	1034	1031	10.0	8.8	2.0	3.0	3.6	4.1	10.4	7.4	3.8	3.8	7.4	3.2	7.2	9.7	4.1	tr	4.3	1.9	7.2	5.3	16.7	
r-Mentha-3,8-diene	1071	1072	1.6	tr	tr	3.36	tr	3.23	1.96	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	
Linalool	1103	1096	1.9	3.7	5.9	0.8	2.6	3.1	4.2	6.7	1.9	8.0	2.2	14.0	4.9	3.7	3.7	9.2	26.1	5.9	3.1	2.7	6.5	
Filifolone	1107		0.1	0.8	3.0	19.7	1.6	0.5	tr	1.6	1.7	2.1	2.3	tr	2.5	1.8	0.2	1.1	1.4	0.4	0.4	0.8	0.8	
Chrysanthone	1131	1127	14.3	24.4	34.1	5.7	30.6	11.2	2.1	26.4	31.0	27.7	38.8	0.2	9.0	36.8	14.9	7.1	15.0	10.0	7.8	3.3	3.3	
trans-Pinocarveol	1137	1139	9.5	2.8	3.3	19.4	5.4	3.3	17.5	4.0	2.2	4.5	3.6	2.0	2.7	1.6	10.8	3.3	5.7	3.2	2.8	2.1	2.1	
Camphor	1153	1146	13.9	19.9	10.3	2.3	10.5	6.9	15.9	2.0	6.8	2.4	2.2	0.8	17.0	4.9	10.4	3.2	6.0	8.1	7.3	18.6	18.6	
r-Menth-3-en-8-ol	1155	1150	1.2	2.8	1.5	0.1	1.5	1.0	2.2	0.3	1.0	0.4	0.3	0.1	2.4	0.7	4.9	0.4	0.8	1.1	1.0	2.6	2.6	
cis-Chrysanthenol	1168	1164	tr	0.1	0.2	3.4	0.2	0.1	0.1	0.1	0.1	tr	0.1	tr	0.2	tr	tr	tr	tr	tr	0.1	0.3	0.3	
Borneol	1166	1169	6.0	6.3	2.1	2.5	1.7	2.1	8.8	2.9	1.5	3.8	5.1	13.6	9.2	9.3	4.8	1.6	4.7	2.3	2.1	11.2	11.2	
Terpinen-4-ol	1176	1177	tr	tr	tr	0.1	tr	tr	tr	0.1	tr	tr	tr	tr	tr	0.1	tr	0.1	tr	0.2	0.3	0.3	tr	tr
r-Cymen-8-ol	1186	1179	tr	0.2	tr	tr	tr	tr	0.1	0.1	0.1	0.2	0.1	tr	0.2	0.1	tr	0.1	0.1	0.1	0.1	0.2	tr	
a-Terpineol	1190	1188	4.0	2.4	0.8	2.7	2.0	0.9	1.8	1.6	tr	1.7	0.6	1.3	1.8	0.5	5.1	1.4	1.9	1.1	1.1	4.0	4.0	
trans-Piperitol	1192	1196	0.1	0.2	0.1	0.1	0.1	tr	0.1	tr	0.1	0.8	0.1	0.3	0.1	0.1	0.2	0.1	0.2	0.1	0.1	tr	tr	
Myrtenol	1197	1195	tr	0.2	tr	tr	tr	tr	tr	0.1	tr	0.1	tr	0.1	tr	tr	tr	tr	tr	tr	0.1	tr	tr	
Verbenone	1204	1204	tr	0.1	0.1	tr	tr	tr	tr	0.1	tr	0.2	0.1	tr	0.1	tr	0.1	0.4	0.1	0.1	0.1	tr	tr	
trans-Piperitol	1207	1208	7.5	0.1	1.1	8.3	4.5	0.1	0.3	1.0	tr	1.5	0.2	tr	0.4	tr	11.7	0.3	1.9	tr	tr	tr	tr	
trans-Carveol	1218	1216	0.2	0.5	0.2	0.2	0.1	19.9	0.1	0.2	tr	0.3	0.2	16.7	0.5	tr	0.4	0.5	8.7	26.6	27.5	0.5	0.5	
cis-Carveol	1231	1229	0.2	0.3	0.4	0.3	0.3	tr	0.1	0.2	0.4	0.5	0.5	tr	0.1	0.4	tr	tr	tr	0.2	0.1	tr	tr	
Pulegone	1243	1237	tr	tr	0.8	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	7.8	tr	0.4	0.1	tr	tr	0.1	tr	
cis-Chrysanthenyl acetate	1266	1265	0.7	0.5	1.4	0.7	1.3	1.5	1.0	3.5	5.1	4.1	3.5	1.2	3.9	3.1	2.0	0.1	1.2	1.3	1.3	0.7	0.5	
Isopiperitenone	1277		0.8	tr	0.1	0.6	0.2	0.4	tr	0.1	tr	tr	tr	tr	0.5	tr	0.3	0.1	0.2	tr	tr	0.7	0.7	
trans-a-Nerolidol acetate	1286	1284	0.2	0.1	tr	0.1	0.1	0.1	0.3	3.7	tr	0.2	0.1	0.1	0.3	0.3	1.3	0.3	tr	0.2	0.1	0.2	0.1	
Bornyl acetate	1289	1288	tr	0.4	0.6	0.1	0.4	0.2	2.9	0.3	0.6	0.6	0.6	tr	0.2	0.4	1.1	0.1	0.2	0.1	0.1	tr	tr	
Lavandulyl acetate	1291	1290	tr	tr	tr	tr	6.4	tr	1.5	10.0	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	
Thymol	1301	1290	0.5	0.4	0.6	1.0	0.1	0.3	0.9	0.7	1.0	1.1	0.7	0.6	0.9	0.2	0.9	1.9	0.8	0.4	0.5	0.6	0.6	
Carvacrol	1311	1299	0.16	0.28	0.28	0.22	0.4	0.2	0.1	0.1	0.1	0.3	0.3	0.1	1.2	0.1	0.3	0.1	0.8	0.1	0.1	0.5	0.5	
trans-Caryyll acetate	1340	1342	0.44	0.58	0.17	0.54	0.4	0.1	0.4	0.1	0.1	1.0	0.1	0.1	0.3	0.1	2.1	tr	0.2	0.2	0.2	0.9	0.9	
Piperitenone	1348	1343	tr	tr	0.3	0.1	0.1	0.1	0.1	tr	tr	0.2	tr	tr	tr	4.7	tr	0.2	tr	tr	0.1	0.1	tr	
neoisodihydro carveol acetate	1354	1359	tr	tr	5.4	tr	tr	16.0	tr	tr	tr	tr	tr	16.7	tr	tr	0.4	0.9	25.2	14.7	16.1	tr	tr	
cis-Caryyll acetate	1364	1367	tr	0.1	0.2	tr	0.4	tr	tr	tr	tr	0.1	0.5	tr	0.2	tr	0.1	0.5	0.1	tr	tr	0.1	tr	
Carvacrol acetate	1374	1372	0.2	0.3	0.1	0.1	0.2	0.1	0.1	0.1	0.7	0.2	0.1	0.1	0.2	0.3	tr	0.1	0.1	0.1	0.2	0.1	0.1	
Geranyl acetate	1381	1381	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	1.08	tr	tr	tr	tr	tr	tr	tr	tr	tr	
(E)-Caryophyllene	1422	1419	0.6	6.0	8.8	3.5	6.0	2.4	0.4	6.2	9.9	9.3	11.2	0.2	1.4	8.5	1.0	1.6	2.9	1.9	1.7	0.8	0.8	
Geranyl propanoate	1478	1477	tr	tr	tr	tr	tr	1.6	tr	tr	2.2	tr	tr	tr	0.2	0.3	0.4	0.5	0.1	tr	1.1	tr	tr	
Bicyclogermacrene	1484	1485	0.2	0.1	0.1	0.7	0.2	0.3	0.4	0.2	0.5	0.2	0.3	0.2	0.3	0.4	0.5	0.6	0.8	0.3	tr	tr	tr	
β-Bisabolene	1500	1500	0.3	0.1	0.1	1.0	0.3	0.1	0.3	0.3	tr	0.2	0.6	0.1	0.3	0.5	0.6	0.2	tr	tr	1.0	tr	tr	
Mintfuranone	1506	1505	tr	tr	tr	tr	tr	1.1	tr	tr	tr	1.8	tr	tr	0.7	tr	tr	tr	tr	0.1	0.2	0.1	0.3	
Sesquiceneole	1508		0.6	tr	tr	0.9	0.8	tr	tr	1.3	0.6	tr	0.2	0.6	0.9	0.1	0.9	0.5	1.1	0.1	0.3	0.2	0.5	
(E)-Nerolidol	1516	1516	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	0.2	tr	tr	tr	tr	0.2	0.6	1.0	tr	
Späthulenol	1566	1563	0.2	0.2	0.3	0.9	0.2	0.9	0.3	0.2	tr	tr	0.8	0.3	0.8	1.0	tr	3.8	10.8	0.5	0.1	0.1	tr	
Caryophyllene oxide	1578	1578	0.2	0.4	0.1	0.6	0.3	0.1	0.4	0.2	0.1	0.4	0.2	0.2	0.2	0.3	tr	0.3	0.2	tr	tr	tr	tr	
Humulene epoxide II	1583	1583	1.2	1.4	0.6	2.9	1.7	2.2	1.6	0.9	1.6	0.4	1.3	1.3	2.2	0.8	3.2	4.7	0.5	1.4	2.3	0.7	0.7	
Caryophylla-4(12),8(13)-dien-5- a-ol	1608	1608	tr	tr	tr	tr	tr	2.1	tr	tr	1.4	tr	tr	tr	tr	tr	tr	tr	tr	0.1	0.2	0.3	0.6	
a-Cadinol	1642	1640	0.2	0.1	0.1	0.2	0.1	0.1	0.1	0.7	0.1	1.2	0.2	0.2	0.3	0.1	0.1	0.1	0.9	1.2	1.7	2.5	1.3	
	1645	1654	0.3	0.1	0.4	0.3	0.3	0.2	0.2	0.4	0.2	0.8	0.5	0.7	0.2	0.2	0.4	0.4	0.9	tr	tr	tr	tr	

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Table 2 (continued)

Component	RI ^a	RI	Accessions (%) ^c																				
			A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15	A16	A17	A18	A19	A20	
		Lit. ^b																					
b-Eudesmol	1656	1650	0.4	1.0	0.4	0.7	2.8	1.4	1.0	0.8	tr	1.5	2.0	0.5	0.7	0.3	0.9	0.5	0.2	0.2	0.4	0.7	
n-Tridecane	2300	2300	0.1	0.1	0.1	tr	0.1	0.1	0.1	0.1	tr	0.1	0.1	0.1	tr	tr	0.2	0.2	0.3	0.7	1.0	0.4	
Total identified (%)			96	95.86	93.35	96.52	93.5	93.93	91.46	93.8	93.7	93.5	92.3	93.08	95.3	93	92.9	87.9	94	94.2	93	93.3	
Monoterpenes hydrocarbons			19.80	10.10	6.40	13.56	6.0	8.83	10.76	12.90	15.80	11.10	9.50	10.90	7.10	12.10	0.40	13.80	3.60	5.10	3.40	16.90	
Oxygenated monoterpenes			72.50	76.26	75.95	72.22	75.50	74.10	75.90	70.90	62.30	67.50	66.10	77.18	81.3	70.10	81.50	54.80	83.80	81.30	78.90	71.60	
Sesquiterpene hydrocarbons			1.10	6.20	9.00	5.20	6.50	3.90	1.10	6.70	12.20	9.70	12.10	1.20	2.00	9.40	2.10	1.90	3.60	2.90	3.10	1.10	
Oxygenated sesquiterpene			2.50	3.20	1.9	5.60	5.40	7.00	3.60	3.20	3.40	5.10	4.50	3.70	4.90	1.40	8.70	17.20	2.70	4.20	6.60	3.30	
Others			0.1	0.1	0.1	tr	0.1	0.1	0.1	0.1	tr	0.1	0.1	0.1	tr	tr	0.2	0.2	0.3	0.7	1.0	0.4	

^a RI, retention indices calculated against *n*-alkanes.^b RI lit., literature retention indices taken from Adams (2007).^c tr, trace (< 0.1).

dihydrocarveol acetate (14.7–25.2%) chrysanthenone (0.2–15%), camphor (0.8–8.1%), and 1,8-cineole (1.9–7.2%). High quantity of chrysanthenone (26.4–38.8%) was representative of A8, A10, A3, A5, A11, A14, and A9 populations and fell into chemotype II. *a*-Pinene (2.6–11.8%), (*E*)-caryophyllene (6–11.2%), camphor (2–10.5%), and lavandulyl acetate (tr–10%) were other main components of chemotype II (Table 3). A previous study revealed that chrysanthenone, lavandulyl acetate, camphor, and *a*-pinene were reported as the main components of *A. wilhelmsii* essential oil (Ghasemi Pirbalouti, 2017).

Chemotype III (A13, A20, A7, A1, A2, A15) was characterized by high concentrations of chrysanthenone (2.1–24.4) and camphor (10.4–19.9%). Other important oil constituents of this ecotypes were *trans*-pinocarveol (2.1–17.5%), 1,8-cineole (tr–16.7%), and borneol (2.5–11.2%).

According to the previous studies, camphor has been reported as the main constituent of *A. wilhelmsii* EO (Afsharypuor et al., 1996; Ghani et al., 2008; Turkmenoglu et al., 2015).

Other *Achillea* species, such as *A. erba-rota* Auct. (Maffei et al., 1989), *A. crithmifolia* Waldst. & Kit (Palić et al., 2003), *A. sieheana* Stapf (Tabanca et al., 2004), *A. conferta* DC. (Saeidnia et al., 2005), and *A. eriophora* DC. (Ghani et al., 2008) showed high content of camphor.

The high amount of linalool (26.1%) followed by *a*-pinene (11.5%) and (*E*)-nerolidol (10.8%) differentiated the Roodbal population as a distinct chemotype (Chemotype IV). This chemotype contained the lowest amount of oxygenated monoterpenes (56.11%) and the highest amount of oxygenated sesquiterpenes (17.21%) compared with the other ones. Linalool was previously reported as one of most abundant components in *A. wilhelmsii* (Javidnia et al., 2004; Alfatehi et al., 2015).

Chemotype V was new and composed by only one population (A4) showing filifolone (19.7%) as the major volatile compound. To the best of our knowledge, no reference is available on the occurrence of this chemotype in *A. wilhelmsii*.

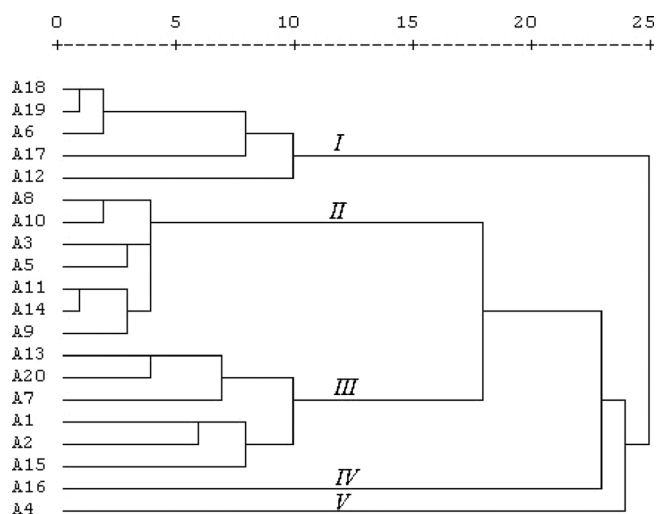
Accordingly, a certain relationship between the distribution of the samples into the clusters and the species type was observed. To sum up, exploring the level of phytochemical diversity in the *A. wilhelmsii* ecotypes collected from southwest climate conditions in Iran provides information of great importance for exploiting their potentials in the food and pharmaceutical industries. Evaluation of inter- and intra-specific variation in essential oil content of medicinal plants and their phytochemical affinity has been the subject of a large number of related studies (Medina-Holguín et al., 2008; Herraiz-Peñalver et al., 2013; Sadeghi et al., 2014; Furtado et al., 2014; Khiyari et al., 2014; Rajabi et al., 2014; Fattahi et al., 2016). It is now widely accepted that genetic and environmental factors as well as agronomic practices (planting and harvesting date, maintenance and post-harvest operations, plant age etc.) are responsible for inter- and intra-species variation in the essential oil compositions and content (Pluhár et al., 2016; Potzernheim et al., 2012; Nurzynska-Wierdak, 2013). In this case, however, the quality and quantity of the essential oils of the plants appear to be controlled more by genetic rather than environmental factors (Marotti et al., 1994; Fahlén et al., 1997). However, as mentioned above, environmental conditions play a substantial role in physiological and morphological characteristics of plants. Due to different climate conditions and other related factors, such as soil regime of different regions, the fluctuations in phytochemical properties of different individuals of the same species may be explainable. Results show that the essential oil analysis can be useful for distinguishing among ecotypes of *A. wilhelmsii*.

3.3. Antioxidant activity

The antioxidant capacity of the *A. wilhelmsii* EOs was measured by spectrophotometric assay using the stable radical 2,2'-diphenyl-1-picrylhydrazyl (DPPH) as a reagent. The IC₅₀ values varied significantly from 129 to 372 mg/ml and were lower than that of the standard compound, BHT (62.6 mg/ml). Results showed that A12 ecotype had

Table 3Major compounds of the essential oils of the twenty Iranian *A. wilhelmsii* K.Koch populations.

Population name	Code	Major compounds (count [%] ^a)
Soreshjan	A1	Chrysanthenone (14.3), Camphor (13.9), 1,8-Cineole (10), <i>trans</i> -Pinocarveol (9.5), <i>trans</i> -Piperitol (7.5), a-Pinene (7.4), Borneol (6)
Borujen	A2	Chrysanthenone (24.4), Camphor (19.9), 1,8-Cineole (8.81), Borneol (6.3), (<i>E</i>)-Caryophyllene (6), a-Pinene (5.4)
Farokhshar	A3	Chrysanthenone (34.1), Camphor (10.3), (<i>E</i>)-Caryophyllene (8.8), linalool (5.9), neoisoDihydro carveol acetate (5.4)
Amirabad	A4	Filifolone (19.7), <i>trans</i> -Pinocarveol (19.4), <i>trans</i> Piperitol (8.3), Chrysanthenone (5.7)
Dehrashid	A5	Chrysanthenone (30.6), Camphor (10.5), Lavandyl acetate (6.4), (<i>E</i>)-Caryophyllene (6), <i>trans</i> -Pinocarveol (5.4)
Bardeh	A6	<i>trans</i> -Carveol (19.9), neoisoDihydro carveol acetate (16), Chrysanthenone (11.2), Camphor (6.9)
Dimeh	A7	<i>trans</i> -Pinocarveol (17.5), Camphor (15.9), 1,8-Cineole (10.4), Borneol (8.8)
Kohyan	A8	Chrysanthenone (26.4), Lavandyl acetate (10), a-Pinene (8.4), 1,8-Cineole (7.4), Linalool (6.7), (<i>E</i>)-Caryophyllene (6.2)
Haydarabad	A9	Chrysanthenone (31), a-Pinene (11.8), (<i>E</i>)-Caryophyllene (9.9), Camphor (6.8), <i>cis</i> -Chrysanthenyl acetate (5.1)
Dezak	A10	Chrysanthenone (27.7), (<i>E</i>)-Caryophyllene (9.3), Linalool (8), 1,8-Cineole (7.4), a-Pinene (7.5)
Aboueshagh	A11	Chrysanthenone (38.8), (<i>E</i>)-Caryophyllene, a-Pinene (7.6), Borneol (5.1)
Gandoman	A12	<i>trans</i> -Carveol (16.7), neoisoDihydro carveol acetate (16.7), Linalool (14), Borneol (13.6), 1,8-Cineole (7.2), a-Pinene (6.6)
Faradonbeh	A13	Camphor (17), 1,8-Cineole (9.7), Borneol (9.2), Chrysanthenone (9), Pulegone (7.8)
Shahrekord	A14	Chrysanthenone (36.8), Borneol (9.3), (<i>E</i>)-Caryophyllene (8.5), a-Pinene (7.2)
Dashtak	A15	Chrysanthenone (14.9), <i>trans</i> Piperitol (11.7), <i>trans</i> -Pinocarveol (10.8), Camphor (10.4), Linalool (9.2), a-Terpineol (5.1)
Roodbal	A16	Linalool (26.1), (<i>E</i>)- Nerolidol (10.8), Chrysanthenone (7.1)
Hajatagha	A17	neoisoDihydro carveol acetate (25.2), Chrysanthenone (15), <i>trans</i> -Carveol (8.7), Camphor (6), Linalool (5.9)
Soodejan	A18	<i>trans</i> -Carveol (26.6), neoisoDihydro carveol acetate (14.7), Chrysanthenone (10), Camphor (8.1), 1,8-Cineole (7.2)
Saman	A19	<i>trans</i> -Carveol (27.5), neoisoDihydro carveol acetate (16.1), Chrysanthenone (7.8), Camphor (7.3), 1,8-Cineole (5.3)
Farsan	A20	Camphor (18.6), 1,8-Cineole (16.7), Borneol (11.2), a-Pinene (6.6), Linalool (6.5)

^a All compounds with a content > 5% of the total oil composition were considered as major compounds; their contents in% are given in parentheses.**Fig. 2.** Average-linkage dendrogram of the 20 *A. wilhelmsii* K.Koch populations resulting from the cluster analysis (based on Euclidean distances) of the essential oil constituents. Chemotype I (*trans*-carveol/neoiso-dihydrocarveol acetate), Chemotype II (chrysanthenone), Chemotype III (camphor/chrysanthenone), Chemotype IV (linalool) and Chemotype V (filifolone/*trans*-pinocarveol) for a detailed description of populations.

the highest activity as a DPPH scavenger with IC₅₀ values of 129 ± 0.011 mg/ml, whereas A3 ecotype had the lowest (372 ± 0.009 mg/ml) (Table 4). Results demonstrated that EOs of *A. wilhelmsii* populations had moderate to weak antioxidant activity as determined by the chemical DPPH radical scavenging assay and that is in agreement with previous studies (Nikvar et al., 2005; Turkmenoglu et al., 2015). Previous researches explained that the antioxidant activities of essential oils can be attributed to their phenolic contents (Nanasombat and Wimuttigol, 2011; Gülçin et al., 2012; Tohidi et al., 2017). The amount of the latter, namely thymol and carvacrol, were low in all *A. wilhelmsii* EOs. Previous studies reported that terpinolene, a-terpinene and g-terpinene (three monocyclic components), and to a lesser degree, sabinene (a bicyclic compound), had considerable antioxidant activity (Choi et al., 2000; Kim et al., 2004; Andrade et al., 2013). However, the presence of the mentioned compounds was not considerable in this study.

Identifying natural products with important radical scavenging activities is very important for the pharmaceutical and food industries in

Table 4Antioxidant capacity of essential oils from twenty populations of *Achillea wilhelmsii*.

Population	IC ₅₀ (mg/ml)
A1	222 ± 0.007 efg
A2	231 ± 0.005 def
A3	372 ± 0.009 a
A4	316 ± 0.022 abc
A5	232 ± 0.005 def
A6	258 ± 0.004 cde
A7	224 ± 0.055 efg
A8	346 ± 0.011 ab
A9	288 ± 0.003 bcd
A10	221 ± 0.004 efg
A11	232 ± 0.003 def
A12	129 ± 0.011 h
A13	164 ± 0.013 gh
A14	184 ± 0.011 fgh
A15	243 ± 0.007 def
A16	323 ± 0.019 ab
A17	236 ± 0.011 def
A18	314 ± 0.046 abc
A19	350 ± 0.032 a
A20	162 ± 0.006 gh

Different letters indicate significant differences between groups (p < 0.05).

order to find potential alternatives to synthetic antioxidant preservatives such as butylated hydroxytoluene (BHT) and others. Factors affecting chemical composition of the plant EOs (e.g. genetic makeup, agroclimatic conditions, and physiological factors) will influence the antioxidant properties of plants, accordingly (Teixeira et al., 2013). Besides the genetic structure of the targeted taxa, together with the environmental conditions, might also profoundly alter the chemical composition of even the same ecotypes, leading to the occurrence of several chemotypes endowed with different bioactivity.

4. Conclusion

The present investigation was aimed at comprehensively studying the essential oil content, composition and antioxidant activity variation in 20 *A. wilhelmsii* accessions in southwest of Iran. In this study, the twenty populations showed a great rate of phytochemical variation and seem to have a high potential for exploitation in breeding programs

useful to the food and pharmaceutical industries. Results revealed that geographic origins and environmental conditions could significantly affect the volatile composition in *A. wilhelmsii*.

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